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Closed-loop growth-rate regulation in fed-batch dual-substrate processes with additive kinetics based on biomass concentration measurement

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ABSTRACT

This paper deals with the design of feeding rates for dual-substrate fed-batch processes where the main control objective is the regulation of the microbial growth rate. To this end, feedback of the growth rate error is incorporated to a biomass proportional dual feeding law. A second-order sliding mode observer is used to estimate the growth rate, so that no additional sensors are required. Stability conditions are derived and robustness against several disturbances such as yield uncertainty, measurement errors and kinetic model mismatch is analytically and numerically evaluated. The advantages of the proposal include: minimal measurement requirements, regulation with fast convergence to the desired growth rate and reduced regulation error in the presence of disturbances.

1. Introduction

Biotechnological processes are applied for the production of metabolites required in the food, pharmaceutical and chemical industries. They also have application in alternative energy generation including production of hydrogen, butane and methane from renewable resources, in the development of new products such as bio-polymers [1], and in remediation of toxic substances such as benzene, phenol and toluene [2,3].

Currently, there exists an increasing interest for multiple-feed processes. These cultures can be considered for enhancing metabolites production [4] and increasing degradation efficiency of toxic compounds [5]. In a multi-substrate process, microbial growth rate is influenced by two or more substrates. These nutrients may perform the same function within the cells, as in the case of two carbon sources, or may be used to fulfil different nutritional requirements, as in the case of a carbon and a nitrogen source [6]. Different types of growth kinetic models have been developed to describe the microbial behavior including additive growth, multiplicative growth, growth in two phases (diauxic growth) and enhanced growth due to a second metabolic pathway [6,7]. From an unstructured point of view, these kinetics lead to different non-linear expressions for the specific growth rate (μ) and the specific rates of substrates consumption.

Fed-batch processes are often preferred to batch ones when maximizing cell or product formation is pursued but chemical reactions are inhibited by some substrate in excess. Also they are a suitable option when some kinetic rate needs to be controlled. In many applications, growth rate regulation is a primary goal since this key variable is closely related to the metabolic state of the microorganism [8]. In fact, controlling growth rate is relevant to avoid formation of undesired by-products when maximizing production of heterologous proteins [9] and to guarantee reproducibility between fed-batch cultivations [10]. Particularly for dual-substrate processes, an additional control objective is to regulate the growth on each of the substrates. This requirement follows, for example, from the relation between substrate consumption and desired product properties [11]. In other cases, feeding of a second nutrient to be degraded or a cheaper carbon source is considered.

With the aim of providing multiple nutrients to the bioreactor, a number of open-loop feeding strategies have been proposed. Many of these strategies are extensions of the feeding laws used in processes with only one-limiting substrate [12,13]. In [14], independent glycerol and methanol exponential feeding rates were designed for increasing productivity of the process. In [5], a dual-exponential feeding law is considered, where the second substrate is fed to increase biodegradation of phenol. In more advanced approaches, the feeding flow rates are adjusted on-line as function of measured variables. In [15], the feeding flow rates increase in proportion to biomass, thus reducing problems associated with nutrient underfeeding or overfeeding. However, since there is no closed-loop w.r.t. growth rate, modelling errors may degrade

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regulation. Then, in [16] a non-linear feeding law was proposed based on the assumption that the individual growth rates are known or can be estimated separately. The fact of distinguishing the growth rates on each substrate may require measurement of more than one substance concentration, for example biomass and one of the substrates.

This paper considers a bioreactor fed with two substrates where the main objective is regulating the growth rate to a given set-point μ_{ref}, which is assumed to be compatible with a desired physiological state of the micro-organism. This set-point is calculated to achieve an specific objective, e.g. to avoid by-products formation, to optimize biodegradation [5], to maintain a metabolic state [10] or to increase the volumetric productivity of a metabolite [14]. The proposed strategy deals with processes that exhibit additive growth kinetics and can be used for supplying two carbon sources. The control law is inspired on closed-loop feeding laws for μ regulation in one-limiting substrate processes [17]. So, the biomass proportional dual feeding rate of previous works is preserved [15,16], but now the proportionality gains are continuously adjusted as function of the total growth rate error. Since there exist growth rate observers based on biomass concentration measurement, already required to implement the biomass proportional feeding strategy, no additional sensors are required for implementing the closed-loop scheme.

The rest of the paper is organized as follows. In Section 2, the model of the fed-batch process is described and the closed loop dual-substrate feeding law is presented. In Section 3, convergence properties are analyzed for different additive kinetics and robustness analysis is performed. In Section 4, the proposed strategy is evaluated under nominal and uncertain conditions. Concluding remarks are given in Section 5.

2. Feeding flow rate design

2.1. Dual-substrate model and control objective

Microbial growth in a fed-batch process under well-mixed condition can be described with the following mass balance model [18]

$$\dot{x} = \mu x - \frac{x}{v}F_1 - \frac{x}{v}F_2, \tag{1a}$$

$$\dot{s}_1 = -\sigma_1 x + \frac{(S_{1in} - s_1)}{v}F_1 - \frac{s_1}{v}F_2, \tag{1b}$$

$$\dot{s}_2 = -\sigma_2 x - \frac{s_2}{v}F_1 + \frac{(S_{2in} - s_2)}{v}F_2, \tag{1c}$$

$$\dot{v} = F_1 + F_2, \tag{1d}$$

where x, s_1 and s_2 are concentration of biomass and substrates (g/L) and v is the working volume. The specific growth rate is given by $\mu(s_1, s_2)$ and the substrate consumption rates are denoted by σ_1 and σ_2. The control inputs are the feeding flow rates of each substrate (F_i) where the inlet concentrations are denoted by $S_{(i)in}$. In this paper, additive growth rate is considered, so the total μ is the sum of two non-negative terms

$$\mu = \mu_1 + \mu_2. \tag{2}$$

The substrate consumption rates can be represented as

$$\sigma_i = \mu_i / y_i, \tag{3}$$

with $y_i > 0$ being the yield of substrate i to biomass.

In this work, it is considered that the primary goal of the feeding applied to the dual-substrate process (1) is the regulation of the growth rate while maintaining a given ratio between substrates s_1/s_2 or between the individual growth rates μ_1/μ_2. That is, it is desired to regulate μ at $\mu_{ref} = \mu_{1r} + \mu_{2r}$, where μ_{ir} stands for the desired growth rate value on substrate i. Therefore, the nominal trajectory for biomass is an exponential profile where the biomass $X(t) = xv$ evolves as

$$X^*(t) = x_0 v_0 e^{\mu_{ref} t}, \tag{4}$$

where x_0, v_0 stand for the initial values of biomass concentration and volume, respectively. Furthermore, it can be observed that:

1. Biomass concentration follows a bounded trajectory given by the logistic function

$$x^*(t) = \frac{\mu_{ref}/\lambda_r}{1 + \left(\frac{\mu_{ref}}{\lambda_r x_0} - 1\right) e^{-\mu_{ref} t}}, \tag{5}$$

with $\lambda_r = \lambda_{1r} + \lambda_{2r}$ being constant gains to be determined later.
2. Volume grows unbounded.
3. Substrate concentrations are constant at (s_{1r}, s_{2r}) for which the desired growth rates (μ_{1r}, μ_{2r}) are obtained.

Growing at constant rate requires feeding the reactor in proportion to biomass population. If biomass and volume measurements are available, flow rates proportional to biomass

$$F_i = \lambda_{ir} x v, \tag{6}$$

can be applied. The proper proportionality gains λ_{ir} can be determined by making the substrate dynamics invariant at s_{ir} after replacing F_i in Eqs. (1b) and (1c) with Eq. (6):

$$\dot{s}_1 = \left(-\frac{\mu_{1r}}{y_1} + (S_{1in} - s_{1r})\lambda_{1r} - s_{1r}\lambda_{2r}\right) x = 0, \tag{7a}$$

$$\dot{s}_2 = \left(-\frac{\mu_{2r}}{y_2} + (S_{2in} - s_{2r})\lambda_{2r} - s_{2r}\lambda_{1r}\right) x = 0, \tag{7b}$$

Since $x(t) \geq \underline{x} > 0$, the solution follows from the linear equations

$$\begin{pmatrix} S_{1in} - s_{1r} & -s_{1r} \\ -s_{2r} & S_{2in} - s_{2r} \end{pmatrix} \begin{pmatrix} \lambda_{1r} \\ \lambda_{2r} \end{pmatrix} = \begin{pmatrix} \frac{\mu_{1r}}{y_1} \\ \frac{\mu_{2r}}{y_2} \end{pmatrix}, \tag{8}$$

and the result is [15]

$$\lambda_{1r} = \frac{\frac{\mu_{1r}}{y_1}(S_{2in} - s_{2r}) + \frac{\mu_{2r}}{y_2}s_{1r}}{S_{1in}S_{2in} - S_{2in}s_{1r} - S_{1in}s_{2r}}, \tag{9a}$$

$$\lambda_{2r} = \frac{\frac{\mu_{2r}}{y_2}(S_{1in} - s_{1r}) + \frac{\mu_{1r}}{y_1}s_{2r}}{S_{1in}S_{2in} - S_{2in}s_{1r} - S_{1in}s_{2r}}. \tag{9b}$$

2.2. Dual feeding law with feedback of the growth rate

The application of the feeding law (6) with gains defined in (9) stabilizes the substrate concentrations at (s_{1r}, s_{2r}). Indeed, global stability is achieved for monotonic kinetics while, naturally, only local stability can be guaranteed when multiplicity occurs due to substrate inhibition. This can be shown with the help of a quadratic Lyapunov function and partial stability theory [15]. The main shortcomings of this control strategy are that convergence speed is very low and steady state errors caused by model uncertainties are high.

In order to speed up convergence and reduce regulation errors due to model uncertainties, closed loop control of the growth rate must be implemented. We propose here to shape the proportionality gains as function of the error in μ regulation as follows

$$F_i = \lambda_{ir}(1 + f_\mu(e)) x v. \tag{10}$$

where

$$e = \mu_{ref} - \mu \tag{11}$$